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Device and method for separating magnetic or magnetizable particles from a liquid

The invention relates to devices for separating magnetic or magnetizable particles from liquids by means of a magnetic field produced by one or more permanent magnets.
The invention further relates to methods for separating magnetic or magnetizable particles from liquids by means of a magnetic field produced by one or more permanent magnets.
The devices and methods can be used, for example, for applications in biochemistry, molecular genetics, microbiology, medical diagnostics and forensic medicine.

Methods based on magnetic separation using specifically binding, magnetically attractable particles are increasingly gaining in significance in the field of sample preparation for diagnostic or analytic examinations. This is true, in particular, for automated processes since it is thereby possible to analyse a large number of samples within a short period of time and to dispense with labour-intensive centrifugation steps. This creates the conditions required for efficient screening at a high sample throughput. This is extremely important for applications in molecular-genetic studies or in the field of medical diagnostics, for example, as it is practically impossible to cope with very large numbers of samples by purely manual handling. Further important fields of application relate to pharmaceutical screening methods for identification of potential pharmaceutical active agents.

The basic principle of magnetic separation of substances from complex mixtures is based on the process of functionalising magnetic particles (magnetizable or magnetically attractable particles) in a specific manner for the intended separation process, that is, they are provided, by chemical treatment, with specific binding properties for the target substances to be separated. The size of these magnetic particles is typically in the range of approx. 0.05 to 500 μm.
Magnetic particles that have specific binding properties for certain substances and can be used to remove these substances from complex mixtures are described, for example, in DE 195 28 029 A1 and are commercially available (e.g. from chemagen Biopolymer-Technologie AG, DE-52499 Baesweiler).

In known separating methods the functionalised magnetic particles are added in a first step ("binding step") to a mixture to be purified which contains the target substance(s) in a liquid promoting the binding of the target substance molecules to the magnetic particles (binding buffer). This causes a selective binding of the target substance(s) present in the mixture to the magnetic particles. Subsequently, these magnetic particles are immobilised on a site of the interior wall of the reaction vessel by employing magnetic forces, that is, a magnetic field, for instance by means of a permanent magnet ("pellet"). Thereafter, the liquid supernatant is separated and discarded, for example by suction or decanting. Since the magnetic particles are immobilised in the manner described, it is largely

prevented that these particles are separated along with the supernatant.

Subsequently, the immobilised magnetic particles are again resuspended. For this purpose an eluting liquid or eluting buffer is used that is suitable for breaking the bond between the target substance(s) and the magnetic particles, so that the target substance molecules can be released from the magnetic particles and separated along with the elution liquid while the magnetic particles are immobilised by the action of the magnetic field. One or more washing steps may be carried out prior to the elution step.

Devices of various types have been described for carrying out separation processes by means of magnetic particles. DE 296 14 623 U1 discloses a magnetic separator provided with movable permanent magnets. As an alternative, it is proposed to move the reaction vessel containing the magnetic particles by mechanical drive means, relative to a fixedly mounted permanent magnet. The device described in DE 100 63 984 A1, which is provided with a magnetic holder and a movable reaction vessel holder, works according to a similar principle.

By using the above-mentioned devices it is possible to immobilise or accumulate the magnetic particles on the interior wall or on the bottom of the reaction vessel as a "pellet". These devices are, however, not suitable for removing the magnetic particles from a reaction vessel. As a consequence it is necessary to exhaust the liquid from each individual reaction vessel by suction in order to separate

the liquid from the magnetic particles. This is a disadvantage as it entails high material consumption (disposable pipette tips). Furthermore, it is unavoidable that individual magnetic particles are also sucked off, thus leading to a high error rate. Other errors can be caused by liquids dripping down, leading to cross-contamination.

DE 100 57 396 C1 proposes a magnetic separator provided with a plurality of rotatable bars that can be magnetised by an electromagnetic excitation coil. By immersing the bar in the liquid containing magnetic particles and withdrawing the bar in the magnetised state, the magnetic particles can be removed from the liquid and, if required, transferred to another reaction vessel where they can be re-released into a liquid, e.g. a wash or elution liquid, by deactivating the excitation coil.
A disadvantage of this device is that the magnetic field produced by the excitation coil is not sufficiently homogenous so that the individual bars - depending on their position within the ring-shaped excitation coil - are magnetised to a different extent. This disadvantage is particularly eminent where a large number of bars is required. In addition, the excitation coil requires a relatively large space, which results in constructional limitations.

Above all, the known devices are not suitable for simultaneous treatment of large numbers of samples as is required in high-throughput applications (e.g. microtitre plates with 364 or 1536 wells).

The object of the invention was therefore to provide devices and methods enabling the separation of magnetic particles from liquids and the transfer of magnetic particles from one liquid into another liquid while avoiding the above-mentioned disadvantages. More particularly, the devices and methods are to be suitable for use in high-throughput processes.

These and other objects are, surprisingly, achieved by a device according to the main claim as well by the methods according to claims 24 to 27, and by the embodiments described in the dependent claims.

Thus, the devices of the invention for separating magnetic or magnetizable particles from a liquid are characterized by the following features:

- The devices comprise two limbs made of a soft-magnetic material; these form - where appropriate together with further components - a magnetic circuit;
- between the two poles of the limbs there is an air gap that is suitable for receiving a container or a plurality of containers;
- a head piece is arranged in a fixed or detachable manner on one of the poles; a magnetizable bar or a plurality of magnetizable bars is/are disposed in a fixed or movable manner on said head piece, in the vertical direction;
- a permanent magnet or a group of at least two permanent magnets is movably arranged on at least one point of the device; the arrangement is such that a magnetic

field can be produced between the two poles and the magnetic field can be activated or deactivated by moving the magnet(s);

- that area of the device wherein the movable magnet(s) is/are arranged in the magnetic circuit (iron circuit) is at least partially surrounded by a material which screens the magnetic field.

The two limbs are made of a soft-magnetic material, for example of soft iron (especially Fe-Ni alloys) or magnetizable steel. The cross-section of the limbs can be square, rectangular, circular or oval, for example; the size of the cross-sectional area depends on the desired cross-sectional area of the magnetic field and may be 20 to 100 cm^2, for example. It is furthermore possible to attach the limbs to a frame or housing made of non-magnetizable material.

The two limbs are typically arranged on top of each other, with the limb carrying the head piece being located above that region of the other limb which serves to receive the liquid containers (i.e. the sample vessels).

The head piece may be arranged so as to be detachable, thus enabling, for example, the replacement of head pieces with different numbers or types (length, diameter; fixed or movable) of magnetizable bars. The number of bars depends on the number of samples, and thereby liquid containers, which are to be treated simultaneously. Microtitre plates are preferably used as containers, especially those with 96, 384 or 1536 wells, so that appropriate head pieces, for example with 96, 384 or 1536 magnetizable bars, are provided

for those cases. Furthermore, also suitable as containers are sample tubes or reaction vessels of a volume of, for example, 0.015 to 100 ml; these can be treated individually or in groups, in each case in combination with magnetizable bars adapted thereto.

The bars, optionally the head piece as well, are also made of a soft-magnetic material, as described above. The length and cross-section thereof are dependent on the intended application purpose, especially on the dimensions of the containers and on the volumes of liquid, and can be varied accordingly.

It is furthermore provided that a replaceable envelope, which can be pulled off, is slipped on each bar in order to avoid cross-contamination between different liquid samples. For this purpose, a special device is preferably provided which enables automatic discarding of the used envelopes and providing and mounting of new envelopes.

By arranging a permanent magnet, which may also be composed of a plurality of individual magnets, a substantially homogeneous magnetic field is produced between the poles of the limbs. In this way it is possible to dispose a larger number of bars, for instance in several rows, with the magnetic field being approximately of the same size at each of the bars; this is of particular advantage with a view to high-throughput processes. A further advantage of the devices according to the invention is that the magnetic particles - in the activated state - accumulate substantially at the tips of the bars.

In accordance with the invention the permanent magnet(s) is/are arranged so as to be movable relative to the magnetic circuit of the device so that the magnetic field between the poles can be alternately activated and deactivated by moving the magnet(s). To this end, the magnet(s) is/are moved within the magnetic circuit, or they are moved into the magnetic circuit and out of it, respectively. This means that the magnetic field between the poles is activated when the permanent magnet(s) is/are in a first position and that the magnetic field between the poles is deactivated when the permanent magnet(s) is are in a second position. In the said second position the magnet(s) is/are preferably outside the magnetic circuit.

The magnetic field is preferably activated and deactivated by moving the magnet(s) within the iron circuit (magnetic circuit) (e.g. by rotation), or by moving the magnet(s) from the outside into the magnetic circuit ("activation") and thereafter out again ("deactivation").

Because of the possibility of activating and deactivating the magnetic field, the device can be used to remove magnetic particles from a first liquid by means of the magnetizable bars and to transfer the particles into a second or further liquid and to release the particles therein. This also allows using the bars, in addition, for other functions, for example as stirring rods.

Basically, any hard-magnetic materials known to the person skilled in the art may be used to produce the permanent magnets, particularly ferrite, Al-Ni-Co alloys and rare

earth magnets (preferably NdFeB); such magnetic materials and magnets are commercially available from various manufacturers.

That area of the device wherein the movable magnet(s) is/are arranged in the iron circuit is at least partially surrounded by a material which screens the magnetic field. A soft-magnetic material may be used as the screening material and/or a material, known to the skilled artisan, which screens magnetic fields, e.g. tinplate or mu-metal. This screening material is arranged around the movable magnet(s) in such a manner that in the deactivated state no magnetic forces are able to act on the containers with sample liquid located in the air gap of the magnetic circuit.
A screening that completely surrounds the region wherein the permanent magnet(s) is/are arranged is especially preferred. More particularly, a short circuit ring may be provided for this purpose.

The device is preferably configured such that if the magnet(s) move(s) within the magnetic circuit or into the same, that area of the device in which the movable magnet(s) is/are arranged in the magnetic circuit is at least partially surrounded by a material which shields the magnetic field.

According to an especially preferred embodiment, the two limbs of the device are connected with each other, at the side opposite the two poles, by a (soft-magnetic) material which is likewise magnetizable, so that a magnetic circuit or a magnetization ring is formed which is completely

closed - with the exception of the air gap between the poles.

The permanent magnet(s) is/are preferably arranged between the two limbs and at their other end (i.e. opposite the poles). If the two limbs are connected with each other, as described, the permanent magnet(s) is/are preferably arranged in or at the region which connects the two limbs. Preferably, the magnet(s) are movably mounted in a recess provided for this purpose in one of the limbs or in the section connecting the two limbs.

To allow movement of the permanent magnet(s) in order to activate and deactivate the magnetic field, the magnet or a group of several magnets may be arranged in a rotatable or tiltable manner in a recess provided for this purpose. By rotating or tilting the magnet, it can moved into a position in which its poles, and thereby its magnetic field, point in the direction of the magnetic circuit, that is, in a direction toward the limbs (activated state, maximal field strength between the poles of the limbs), or it can be moved into another position in which the magnetic field emanating therefrom is substantially perpendicular to the aforementioned direction (deactivated state). The magnet(s) may also be rotated or tilted into positions therebetween to achieve a field strength between the poles of the limbs which is lower than the maximum value.

Alternatively, it is also possible to mount the magnet(s) in a displaceable manner such that the magnet(s) can be

moved into the magnetic circuit by displacing the same (activation), or removed therefrom (deactivation).

The movement (e.g. tilting, rotating, displacing) may be accomplished either manually in a direct or indirect manner, or by means of one or more electric motors, or by pneumatic or hydraulic means; combinations of these means are also possible. The drive means may comprise further means known to those skilled in the art, such as a linkage or a gear unit.

According to a preferred embodiment, the extent of the movement of the permanent magnet(s) is predeterminable. In this manner, it is possible to set the magnetic field strength to a specific value, depending on the given application purpose. This can be accomplished, in particular, by predetermining and adhering to a certain tilting or rotation angle, or a certain displacement distance.

According to a further embodiment, the headpiece, which bears the magnetizable bars, is mounted so as to be movable. In particular, the headpiece may be movable in the horizontal plane. In that case, the drive means (e.g. electrical, pneumatic and hydraulic), gear units, linkages and the like are connected with the headpiece, so that the headpiece can be used for carrying out shaking movements (e.g. circular movements or movements as those of an orbital shaker).

It is further preferred for the said magnetizable bar(s) to be rotatably (around the longitudinal axis thereof) mounted

on the respective head piece and that it/they can be rotated during the treatment of a magnetic particle-containing liquid in order to accomplish intermixing or to accelerate the separation of the particles from the bars. Rotation is preferably accomplished by electromotive means.

To separate magnetic particles, liquids containing such particles are introduced in the air gap of the device, below the magnetizable bars; for this purpose, containers of the type mentioned above can be used. Preferably, at least one holding device is provided for this purpose which can be positioned below the bars, so that the bars are oriented towards the openings of the containers. This holder may be configured, for example, in the form of a holder plate.

Further preferred are embodiments wherein the holding means is movable in an essentially horizontal plane in one direction or a plurality of directions; alternatively or in addition thereto, the holder may be movable in the vertical direction. The movement is preferably accomplished by means of an electromotive drive or by pneumatic or hydraulic means, or by combinations of these means.

In particular, the holding devices may also be configured such that they can be used for carrying out shaking movements. The constructional measures required therefor are basically known to the person skilled in the art. It is furthermore provided that both the head piece and the holding device may be movable and utilised to carry out shaking movements. It is thereby possible to achieve an especially

effective intermixing of the sample liquid when the bars are immersed therein.

It is furthermore preferred that an open-loop control device or a closed-loop control device be provided by means of which the vertical movement of the holding device(s) can be adjusted or controlled such that an upward movement thereof causes the bars (7) to be immersed in the containers (10), which are filled with liquid.

In particular, the above-mentioned holding device may be a component of a program-controlled laboratory robot system; preferably, the holding device is adjusted such that a plurality of individual ones of the said containers or groups of such containers, particularly microtitre plates, are alternately moved into a position below the said bars and subsequently, after a predeterminable time interval, again into a position which is outside the region below the bars. This allows a high sample throughput.

According to a further, particularly preferred embodiment of the invention, a program-controlled processor is associated to the device and is connected therewith. In this way, at least one of the following functions of the device can be open-loop controlled or closed-loop controlled, or at least two of the functions mentioned below can be combined with one another:

- movement of the permanent magnet(s) to activate and deactivate the magnetic field, particularly the duration of the activated and deactivated phases, as well as magnetic field strength;

- rotation speed and duration of rotation in the case of rotatable bars;
- movement of the head in a horizontal plane, particularly duration, frequency and amplitude of a shaking movement;
- movement of the holding device(s) to position the container(s) or groups of containers alternately below the bars and subsequently removing them from that position, particularly the velocity and frequency of the movements, as well as the dwell time of the holding device below the bars;
- vertical movement of the holding device to immerse the bar/the bars in the liquid of the container(s) and remove the same therefrom; particularly immersion depth, duration and frequency;
- if provided, rotation or shaking movement of the holding device(s), particularly rotation speed, rotation amplitude and intervals between the individual operation phases.

The devices according to the invention may advantageously be combined with other devices for automatic treatment of sample material. Furthermore, two or more of the devices according to the invention may be arranged side by side and combined with one another.

The invention therefore also encompasses devices of the type described above to which one or more of the following means are associated, the functions of said means being coordinated with the functions of the device by means of a joint control:

- one or more thermostattable heating or cooling means;
- one or more pipetting stations for metered addition of liquids, especially reagents;
- one or more suction means for exhausting liquid from the containers;
- one or more means for shaking or intermixing the liquids contained in the containers;
- analytic apparatuses, particularly for photometric measuring or luminescence detection.

The invention further comprises methods for separating a target substance from a substance mixture present in liquid form. These methods generally comprise the following steps:

a) addition of magnetic or magnetizable particles that have specific binding properties in relation to the target substance;
b) placing a pre-determined volume of the mixture in the air gap between the two poles of a magnetic circuit and immersing a magnetizable bar into the mixture, the said bar being connected with one of the poles of the magnetic circuit, and the magnetic field being initially deactivated;
c) activating the magnetic field by changing the position of a permanent magnet arranged in or on the magnetic circuit, whereby the bar is magnetized and the particles accumulate substantially at the lower end of the bar; subsequently, the bar is removed from the first mixture of liquids, along with the particles which adhere thereto;
d) immersing the bar, together with the particles adhering thereto, into a predetermined volume of a liquid

that causes the elution of the target substance from the particles;

e) removing the bar from the elution liquid, whereby the particles continue to adhere to the bar and are thereby separated from the liquid.

To improve purity and yield, it may be advantageous to release the particles into the liquid, following step d, by deactivating the magnetic field, to mix the liquid and subsequently to re-accumulate the particles on the bars by activating the magnetic field. Intermixing can be accomplished, for example, by rotation of the bars or by agitating the holder or/and the head piece.

Furthermore, the above-described method may optionally comprise one or more washing procedures; such a washing process may, for example, follow step c) and be carried out as follows:

- immersing the bar, together with the particles adhering thereto, into a pre-determined volume of a wash liquid;
- deactivating the magnetic field by an opposite change of the position of the permanent magnet, whereby the particles are released into the liquid;
- mixing;
- activating the magnetic field by changing the position of a permanent magnet arranged in or on the magnetic circuit, whereby the bar is magnetized and the particles accumulate substantially at the lower end of the bar;

By using one of the above-described devices according to the invention it is possible to carry out the above-mentioned methods in a particularly simple and rapid manner. The devices and methods according to the invention can be used to particular advantage for the application fields mentioned at the outset, especially for high-throughput methods.

In the following, the invention will be illustrated, by way of example, by means of the schematic drawings appended hereto. The meaning of the reference numbers used is the same in all of the drawings, unless otherwise stated. Since the drawings are merely schematic representations, the actual size ratios may vary therefrom.

Fig. 1A and 1B depict an embodiment of a device according to the invention, in side view. The device (1) has two magnetizable limbs (2, 3) of a magnetic circuit, said limbs being connected with each other in the region (6). At the opposite end of the limbs are the two poles (4, 5), with an air gap (12) located therebetween. The pole (4) of the upper limb (2) carries a head piece (8) with bars (7) attached thereto. Below the bars there is a holding device (11) which is connected with the pole (5) of the other limb (3) or is at least in contact therewith. On the holding device, there is arranged a sample container (9) having a plurality of depressions (10) for receiving liquid samples - for example, fixed on the holding device (11) in a detachable manner.

On the side opposite the air gap (12), in the region (6) connecting the two limbs, there is a recess (16) wherein a bar-shaped or cuboid permanent magnet (15) is rotatably arranged. Around the region of the permanent magnet there is arranged a short circuit ring (20) (the latter is represented by dashed lines in the area of the rotatable magnet). Fig. 1A shows the device in the deactivated state; the position of the permanent magnet (15) is substantially perpendicular to the direction of the magnetic circuit; the magnetic field of the permanent magnet is guided into the short circuit ring (20).

Fig. 1B shows the same device in the activated state. The position of the permanent magnet (15) points substantially in the direction of the magnetic circuit. Thereby, a magnetic field is formed between the poles (4, 5) and thus also at the ends of the bars (7); this magnetic field can be used to attract magnetic particles.

Fig. 1C shows a section of the device shown in Fig. 1A/B in the plane indicated by the dashed line a (Fig. 1B). Arrows (17) schematically show the direction of the magnetic field in the activated state.

Figs. 1D and 1E show, likewise in schematic side view, a further embodiment of the devices according to the invention, wherein the magnet used has a flat cuboid shape and the poles are located at the two large side surfaces. Figure 1 D shows the activated state (the magnetic field runs in the direction of the iron circuit) and Fig. 1 E shows the deactivated state. The position of the short circuit

ring (20) is merely outlined. The other elements shown in Figs. 1A, 1B have been omitted for the sake of simplification.

Fig. 2 and Fig. 3 show further construction variants of the device according to the invention, likewise in side view.

Fig. 4 shows the device (1) of Fig. 1A/B in plan view; in this way the ring shape of the short circuit ring (20) is visible. In the embodiment shown, the short circuit ring (20) is configured such that it does not completely abut the magnetic circuit but leaves a cavity (22). This facilitates or enables access to the rotatable magnet (15). The short circuit ring (20) can be composed of two halves (20a, 20b) or a plurality of parts, as indicated by the dashed line 21, in order to facilitate assembly and disassembly.

Fig. 5 shows an embodiment of the device according to the invention (likewise in side view), wherein a displaceable (double arrow) permanent magnet (15) is provided in the recess (16). Fig. 5 shows the activated state, where the permanent magnet causes a magnetic field to be formed between the poles (4, 5). For deactivation, the magnet is displaced outwardly, out of the magnetic circuit of the device (1).

Fig. 6 shows a modification of the device shown in Fig. 1A/B, wherein the two limbs (3, 4) are of different length.

Figs. 7A to 7D show different views of a particularly preferred embodiment, wherein a magnet (15) is placed on a support (40) which is rotatable in a horizontal plane,

about axis Y. The magnet (15) can thereby be moved into the magnetic circuit (iron circuit) by rotating the support (40) (activated state, Figs. 7C, 7D), or out of the region of the magnetic circuit (Figs. 7A, 7B). The short circuit ring (20), which is not represented in these Figures (Figs. 7A to 7D) is provided with an appropriate recession in the region of the support (40), or the shielding material is provided in an incomplete manner on that side of the device. The support (40) is preferably provided in the form of a turn table, or possibly as a rotatable arm, moved by means of known drive means. Optionally, two or more magnets can be attached on the support.
Figs. 7A and 7C show a section in the surface of the turntable Y; Figs. 7B and 7D show the same device, respectively, in plan view.

Fig. 8 shows an embodiment of the device (1) according to the invention, in side view; in this case, the two limbs (2, 3) are not connected with each other by a common region (6). The rotatable magnet (15) is arranged between the two limbs (2, 3), on the side opposite the air gap. The short circuit ring (20) is represented in cross-section.

Fig. 9 shows the front view of the upper limb (4) of a device according to the invention, with the head piece (8) and the bars (7) attached thereto. Below the bars there is arranged a holding device (8), on which a plurality of containers (10) is arranged in rows. The holding device can be moved in the horizontal plane in various directions, as well as upwards and downwards (arrows).

Fig. 10 (a-d) shows, in longitudinal section, examples of different shapes of the magnetizable bars (7). The particles which have been attracted under the influence of the magnetic field are indicated at (30). Fig. 9d shows a bar that is provided with a replaceable envelope (25).